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THE ANTIFUNGAL ACTIVITY OF SKIN SECRETION AND ITS EXTRACT OF INDIAN TOAD *BUFO MELANOSTICTUS*

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ABSTRACT

The present study was aimed to determine the antifungal activity of skin secretion and its extract of Indian toad Bufo melanostictus (Schneider). Amphibian skin possesses a wide variety of biologically active substances that can be used in therapeutics. For the present investigation, the toads and frogs were induced to release a skin secretion using electrical stimulation by using platinum electrodes rubbed over the moistened dorsal skin surface for 10sec. The collected skin secretions were filtered-sterilized, freeze dried and subjected to antifungal assay. Antifungal activity of the toad skin secretion was tested on A sthana-Hawkers agar medium plates seeded with the species of Aspergillus Niger, Penicillium notatum through well diffusion technique by using minimum inhibition concentration (MIC) method. An inhibition was observed with 40µl/ml of toad skin secretion. According to our observations, the skin secretion and its extract of toad have shown antifungal activity against two strains of A. niger and P. notatum. Both the skin secretion and its extract have shown nearest inhibition zones i.e. viz., 23mm, 27mm and 25mm, 23mm respectively, therefore the toad skin secretion and its extract possess has the potential to be developed as a source of antifungal agents.

KEYWORDS: Bufo Melanostictus, Skin Secretion, Asthana-Hawakers Agar Medium, Antifungal Agents, Aspergillus Niger & Penicillium Notatum

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Original Article

INTRODUCTION

Fungi are eukaryotes, which are an important cause of infections to plant, animal and human diseases. Fungal infections have been reported to increase gradually in individuals with reduced immune system. Treatment is limited against fungal infections due to effective antifungal agents are not available compared to antibacterial agents (**Katerere *et al.*, 2013, Barlian *et al.*, 2011**). In some cases fungi are indirectly responsible for allergic (or) toxic disorders because of the production of mycotoxins(**Marco Dalla Rizza 2011**). Pathogens such as chytrid fungus and irido viruses also affect different species of amphibians (**Woodhams2005**). Amphibians can live in both terrestrial and aquatic ecosystems, in such a manner that its skin plays an active role in physical and physiological activities(**Clarke1997**). Toad skin contains a wide range of bioactive compounds like alkaloids, biogenic amines, proteins and peptides. These bioactive compounds can develop the defense mechanism against the predators, bacteria, fungi and other microorganisms (**Simmaco *et al.*, 1998**). Frog skin secretion can be utilized for the benefit of human beings, with its anti-bacterial, anti-fungal, anti-protozoal, anti-diabetic and some other therapeutic properties (**Gomes *et al.*, 2007**). **Conlon and Sonnevend** (2011) have reported that the skin secretion of many Anurans (toads & frogs) contain peptides with antifungal activity. These chemical substances are secreted from the granular glands of frog which are located in the dorsal region of skin **Abishek Grag(2007)**, **Rollins**

Smith *et al*., (2005) found that most of the Amphibian species that they have tested had one (or) more peptides with potent activity against fungi. Now a days scientists are exploring the therapeutic potential of skin secretions of toad and its extracts (**I. Made Artika *et al.*,2015, Wulandari *et al*., 2013**).

In view of the above we have under taken the present investigation to screen the antifungal activity of the skin secretion and its extract of *B. melanostictus*.

MATERIAL & METHODS

Collection of Toad and Toad Skin Extract (TSE) Preparation

Adult live toads of *Bufo melanostictus* 40-50 gm were collected from the vicinity of Kakatiya University hostel buildings and maintained in well ventilated glass box and some insects were given as feeding. animals was pithed and their skin was separated from the body except parotid gland. The skin was kept in methanol at room temperature for 30 days. The supernatant was centrifuged and was pooled. It was evaporated to dryness in rotary evaporator and the extract was kept at room temperature (28°C) in a desiccators. The Toad Skin Extract (TSE) was dissolved at a definite concentration in normal saline (0.9%) until used. **(Manika Das *et al*., 1996).**

Collection of Toad Skin Secretion

The skin secretions were obtained from the toads by gentle electrical stimulation (4-ms pulse width, 50Hz, 5V) using platinum electrodes rubbed over the moistened dorsal skin surface for 10s. Secretions were washed off into a glass beaker, using distilled water. The resultant secretions were freeze-dried. Approximately 50 mg, dry weight of skin secretion was obtained. This procedure was carried out in accordance with the UK Animals Scientific Procedures (Act 1986). It is a non-invasive technique causing no distress to the frog. **(L Marenah, P R Flatt, D F Orr, C Shaw** and **Y H A Abdel-Wahab)**.

Antifungal Activity

Fungal suspension was swabbed on the surface of the solidified Asthana-Hawkers agar medium. Now 40µl of each extract, secretion was added into the wells and plates were incubated at 28^0 C for 7days. A zone of inhibition was measured with Hi-media antibiotic scale and experiment was repeated three replicates. Inhibition zones were measured and tabulated. All the qualitative and quantitative chemicals were supplied by Himeda Laboratories Pvt. Ltd. Mumbai.

RESULTS

Results obtained from the present investigation are presented in table1, plate 1&2 and figure1. The highest inhibition zone (table 1) against *Aspergillus* 25±4.2mm and lowest activity towards *Penicullium* species 23±2.0mm was shown by skin secretion. The skin extract showed highest inhibition activity zone against *Aspergillus* (23± 4.9mm) and the lowest inhibition zone activity towards *Penicillium* species (21± 2.5mm). Both skin secretion and its extract have showed inhibition zones with nearest values like viz., 25±4.2mm, 23±2.0mm, 23±4.9mm and 21±2.5mm respectively. Thus, from our present investigation, it is evident that both skin secretion and its extract have the potency to inhibit the growth of all fungal strains tested.

Table 1: Antifungal Activity of Toad Skin Secretion and Its Extract

Microorganism	Skin Secretion	Skin Extract
Penicillium	23 ±2.0mm	21 ±2.5mm
Aspergillus	25 ±4.2mm	23 ±4.9mm

MIC = Minimal Inhibitory Concentration, The Lowest Concentration at which no growth was detectable.

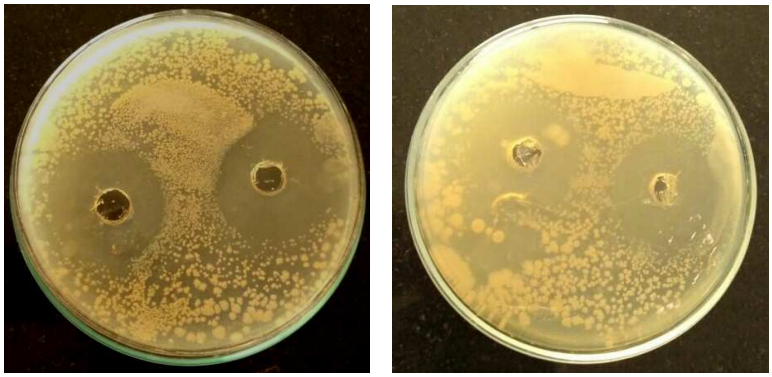

Plate 1&2: Zone of Inhibition of Toad Skin Secretion and its Extract

Penicillium Aspergillus

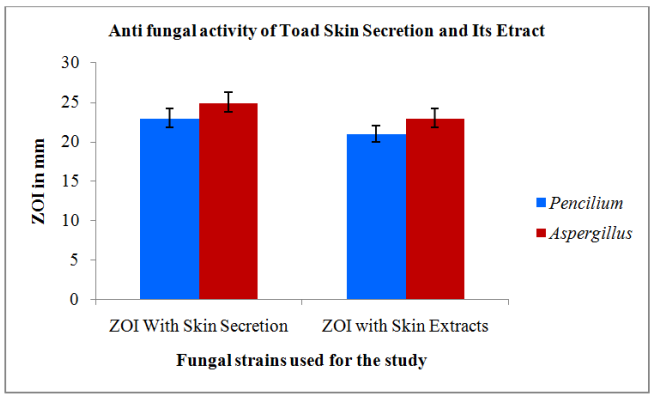

Figure 1: Anti Fungal Activity of Toad Skin Secretion and Its Extract

DISCUSSIONS

Amphibians exist in a microorganism rich environment, and as a result, they produce potent antimicrobial peptides as defence (**Barra D 1995**). Over past two decades Amphibian skin secretions have become a pivotal model for the discovery of new bioactive molecules with potential therapeutic activities such as antimicrobial, anti-diabetic, anti-neoplastic and sleep inducing properties; thus attention has been increasingly focused upon Amphibian skin peptides as a potential therapeutic agent (**I. Made Artika 2015**). Previous studies have reported that the skin secretions from *L. cruentata* and *R. margaritifer* showed antifungal activity (**I. Made Artika, Sabrina Pinontoan and Mirza Dikari Kusrini, 2015**). The ability of bioactive compounds in the skin secretions to inhibit the growth of fungus **(Grag *et al.*, 2007, Afsar*et al.*, 2011, Barlian *et al.*, 2011)**. **Katerere *et al.***, (2013) also reported that skin secretions of frogs (*Amietia fuscigula, Strongylopus grayi* and *Xenopus levis*) and toad (*Amietophrynus pantherinus*) from south Africa showed antifungal activity against *Canadida albicans, Fusarium, Verticelliodes* and *Aspergillus flavus.*

Thus, from our present investigation, our results report that the skin secretion and its extract have got therapeutic properties as they have shown antifungal property.

CONCLUSIONS

From our present results, it can be concluded that the skin secretion and its extracts of toad *B melanostictus* has the potential to develop as a source of antifungal agents and can be used in the treatment of various diseases. This suggests that the studies have to be focused on frog skin secretion and its extract and further studies should be carried out using different approaches depending on the purpose of the study.

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REFERENCES

1. *Afsar, B.M. and Kalyoncu, F. (2011). Antimicrobial activity in the skin secretion of brown frog, Rana macrocnemis (Boulenger, 1885) collected from Turkey. Acad. J., 6: 1001-1004. DOI: 10.5897/SRE10.237*
2. *Barlian, A., Anggadiredja. K and A. Kusumorini.(2011). Damage in fungal morphology underlies the antifungal effect of lyophilisate of granular gland secretion from duttaphrynus melanostictus frog. J. Biol. Sci., 11, 282-287.*
3. *Barra, D& Simmaco, M. (1995). Amphibian skin: A promising resource for antimicrobial peptides. Tibtech, 13,205–209. http://dx.doi.org/10. 1016/S0167- 7799(00)88947-7.*
4. *Marenah, B. L., Flatt, P. R., Orr, D. F., Shaw, C and Abdel-Wahab Y. H. A. (2006) Skin secretions of Rana saharicafrogs reveal antimicrobial peptides esculentins-1 and-1B and brevinins-1E and -2EC with novel insulinreleasing activity. Journal of Endocrinology, 188, 1–9.Society for Endocrinology Printed in Great Britain.*
5. *Clarke, B. (1997)The natural history of amphibian skin secretions, their normal functioning and potential medical applications, Biol Rev Camb Philos Soc., 72, 365–379.*
6. *Conlon, J.M. and Sonnevend. A. (2011). Clinical applications of amphibian antimicrobial peptides. J. Med. Sci., 4: 62-72. DOI: 10.2174/1996327001104020062.*
7. *Garg, A.D., Kanitkar, D. V., Hipargi, R.V and Gandhare. A.N.(2007). Antimicrobial activity of skin secretions isolated from Indian toad, Bufo melanostictus Schneider 1799. Nature Precedings, DOI: 10.1038/npre.2007.1204.1*
8. *Gomes. A, Giri. B, Saha. A, Mishra. R, Dasgupta. S.C, Debnath. A,(2007a). Bioactive molecules from amphibian skin: their biological activities with reference to therapeutic potentials for possible drug development. Indian J. Exp. Biol., 45, 579-593.*
9. *Made Artika. Iet al.,(2015). Antifungal Activity of Skin Secretion of Bleeding Toad Leptophryne Cruentata and Javan Tree Frog Rhacophorus Margaritifer American Journal of Biochemistry and Biotechnology, 11 (1): 5.10. gagrepatil, v. a., & vanmare, d. assessment of fungal pathogens of capsicum in nashik district.*
10. *Katerere, D.R., Dawood, A., Esterhuyse, A. J., Vismer, H.F and Govender. T. (2013). Antifungal activity of epithelial secretions from selected frog species of South Africa. Afr. J. Biotechnol. 12: 6411-6418. DOI: 10.5897/AJB2013.12036.*
11. *Lazarus. L and Attila M. (1993).The toad, ugly and venomous, wears yet a precious jewel in his skin. Prog Neurobiol.41:473–507. http://dx.doi.org/ 10.1016/0301-0082(93)90027-P*
12. *Simmaco. M, Mignogna. G and Barra. D. (1998). Antimicrobial peptides from amphibian skin: what do they tell us?*

Biopolymers 47 (6), 435–450.

13. *Manika Das, Auddy. B and Gomes. A. (1996). Pharmacological Study of the Toad skin extract on experimental animals Indian Journal Of Pharmacology, 28, 72-76.*
14. *14 Priya, T. (2017). Antimicrobial Activity of Evovulus Alisinoids (L) Extract with Different Organic Solvents in Pathogenic Bacteria and Fungal Species.*
15. *Marco Dalla Rizza. (2011). Antifungal activity of medicinal plant extracts against phytopathogenic fungus alternaria spp. Chilean Journal of Agricultural Research 71(2),231-239.*
16. *Nicolas. P., Mor. A. (1995). Peptides as weapons against microorganisms in the chemical defense system of vertebrates. Annu Rev Microbiol.49,277–304. http://dx.doi.org/10.1146/annurev.mi.49.100195.001425, PMid:8561461*
17. *Rollins-Smith, L.A., Reinert, L.K., O'Leary, C. J., Houston. L. E and Woodhams, D. (2005). Antimicrobial peptide defenses in amphibian skin. Integr. Comp. Biol., 45: 137-142. PMID: 21676754*
18. *Tyler, M.J., Stone, D. J. M and Bowie. J. H. (1992) A novel method for the release and collection of dermal, glandular secretions from the skin of frogs. J Pharmacol Toxicol Methods. 28(4),199–200. http://dx.doi.org/10.1016/1056-8719(92)90004-K*
19. *Zaidi, R. K., & Pathak, N. (2014). Antifungal activity of plant extracts and Trichoderma sp. against seed fungi of Cowpea. International Journal of Botany and Research.[IJBR](Trans&Stellar). Pages, 51-60.*
20. *Woodhams, D. C, and Alford. R. A.(2005). Ecology of Chytridiomyco- sis in rainforest stream frog assemblages of tropical Queensland. Conservation Biology 19:1449-1*
21. *Wulandari, D.R., Ibrohim. M. H and Listyorini. D.(2013). The observation of frog species at State University of Malang as a preliminary effort on frog conservation. J. Tropical Life Sci., 3: 43-47.*